00766.000044. PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
YOSHIKI SASAI, ET AL.	)	Examiner: Magdalene K. Sgagias, Ph.D.
Application No.: 09/855,587	)	Group Art Unit: 1632
Filed: May 16, 2001	)	Confirmation No. 1416
For: PROCESS OF INDUCING DIFFERENTION OF EMBRYONIC CELL TO CELL EXPRESSING NEURAL SURFACE MARKER USING OP9 OR PA6 CELLS (AS AMENDED)	)	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. §1.132

Sir:

I, Dr. Yoshiki Sasai, do hereby declare as follows:

1. I received my MD from the Kyoto University School of Medicine in 1986 and subsequently performed internships in both general practice and emergency medicine.

2. I obtained a Ph.D from the Kyoto University School of Medicine in 1992. My Ph.D was awarded for work on neural specific transcriptional regulators.

3. I took a postdoctoral fellowship in the Dr. Edward De Robertis laboratory at the UCLA School of Medicine in 1993. The De Robertis laboratory focuses

on the molecular biology of vertebrate gastrulation, e.g., the coordinated development of groups of cells forming the three germ layers culminating in the determination of the main regions of the embryo: the head, trunk and tail. I remained at UCLA in the De Robertis laboratory until I was appointed associate professor at the Kyoto University School of Medicine in 1996.

4. I assumed a professorship at the Kyoto University Institute for Frontier Medical Sciences in 1998.

5. I was appointed Group Director at the Center For Developmental Biology of RIKEN in 2000 and have maintained that position to date.

6. I serve on the editorial boards of Neuron, Genesis, and Developmental Dynamics.

7. I am an inventor of the present application and am familiar with its specification. I am also familiar with the prosecution of the present application, including the Examiner's contention the specification does not literally teach culturing embryonic stem cells *in vitro*, in the presence of OP9 or PA6 stroma cells and in the absence of retinoic acid, without forming embryonic body to produce a cell expressing a neural crest or neural tube marker. The Examiner is incorrect, as I explain below.

8. One of ordinary skill in the art of the present application has received a Ph.D in work on differentiation processes and regulation, and has completed at least five years relevant post-doctoral research.

9. I am one of ordinary skill in the art of the present application.

10. Based upon my review of the specification, it is my opinion that one of ordinary skill in this art would recognize that the specification at page 45, lines 1-6 states

> (6) Culturing without using retinoic acid
>
> According to the method for inducing differentiation of the present invention, it is preferred to culture the embryonic stem cell without using retinoic acid in the culturing step of the cell under non-aggregation conditions.

11. In conformity with the teaching at page 45 discussed above, Example 1 shows culturing embryonic stem cells *in vitro*. Example 1 does not say that retinoic acid is not utilized, but it is conventional in this art when explaining culture processes to explicitly recite all conditions and materials employed. That is to say, those of ordinary skill herein recognize that if a material is not mentioned, it was not used. Thus, Example 1 in fact teaches to one of ordinary skill in this art culturing embryonic stem cell EB5 with stroma cell PA6 (specification page 92, lines 19-25). None of the discussion of Example 1 in its entirety from specification page 90, line 23 to page 95, line 6 mentions retinoic acid. Therefore, as one of ordinary skill in this art, I understand from Example 1 that retinoic acid was implicitly not used therein. Moreover, as one of ordinary skill in this art I believe literal support for not using retinoic acid is found in Example 1 in combination with the discussion above-noted at specification page 45, lines 1-6.

12. Also, in conformity with the teaching at page 45 discussed above, Example 14 shows culturing embryonic stem cells *in vitro*. Example 14 does not say that retinoic acid is not utilized but again it is conventional in this art when explaining culture processes to explicitly recite all conditions and materials employed. That is to say, those

of ordinary skill herein recognize that if a material is not mentioned, it was not utilized. Thus, Example 14 teaches to one of ordinary skill in this art culturing embryonic stem cell EB5 with stroma cell PA6 (specification page 115, lines 4-13). None of the discussion of Example 14 in its entirety from specification page 114, line 30 to page 117, line 28 mentions retinoic acid. Therefore, as one of ordinary skill in this art, I understand from Example 14 that retinoic acid was implicitly not used therein. Moreover, as one of ordinary skill in this art, I believe literal support for not using retinoic acid is found in Example 14 in combination with the discussion above-noted at specification page 45, lines 1-6.

13. I am a named author of Generation of dopaminergic neurons and pigmented epithelia from primate ES cells by stromal cell-derived inducing activity, *P.N.A.S.*, Vol. 99, No. 3 (2002) 1580-85, which was brought to the Examiner's attention in the July 16, 2003 amendment in this application. In the November 5, 2010 Advisory Action, the Examiner states

> The Examiner has reviewed said PNAS paper (PNAS, 99(3):1580-1585, 2002), which is Applicant's own paper and there is no teaching of culturing primate ES cel in the absence of retinoic acid.

Accordingly, to clarify the record, I hereby confirm that retinoic acid was not utilized in culturing ES cells in the experiments reported in that PNAS paper.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dr. Yoshiki Sasai

Date: November 24, 2010